TREAT-NMD Activity A07: Accelerate preclinical phase of new therapeutic treatment development

Work package 7.4: Develop standardised protocols and procedures for harmonising and accelerating pre-clinical studies (including standardised data analysis)

SOP Title
Measuring Tibiotarsal Joint Angle to Assess Contractures In Vivo

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1. **OBJECTIVE**

   Most DMD natural history studies have included measurements of muscle strength, joint contractures, and timed function tests. Results from these tests are used to track disease progression and offer insight on clinical milestones, such as the loss of ambulation and the need for ventilatory support. Both muscle weakness and joint contractures contribute to postural instability and ultimate loss of ambulation.\(^1\) Joint angles can be objectively measured to determine the severity of contractures.

2. **SCOPE AND APPLICABILITY**

   Contracture and muscle strength scores generally correlate and deteriorate synchronously over time.\(^2\) Joint contractures occur due to an imbalance in the strength of agonist and antagonist muscles. Weakness of the antagonist extensor muscle correlates highly with flexor contracture severity. Thus, as opposing extensor muscles weaken, flexor contractures worsen.\(^2\) GRMD dogs characteristically have a plantigrade stance and gait as evidenced by hyperextension of the carpal joints in the thoracic limbs and flexor contractures at the tibiotarsal joints in the pelvic limbs. We have measured the tibiotarsal joint angle to objectively demonstrate the severity of contractures.\(^3\) Consistent with DMD, tibiotarsal joint angle and extensor force correlate strongly (see separate tibiotarsal joint force protocol).\(^4\) Dogs with stronger tibiotarsal joint extensors have more normal joint angles. In addition, tibiotarsal joint flexor force and joint angle correlate inversely, suggesting that flexor muscle functional hypertrophy can contribute to contractures. Taken together, these findings suggest that tibiotarsal joint angle can be used as an outcome parameter in preclinical studies.

3. **CAUTIONS**

   Dogs must be anesthetized. Otherwise, the only equipment required is a goniometer. See METHODS and EVALUATION AND INTERPRETATION OF RESULTS (below) for guidance on interpretation.

4. **MATERIALS**

   Dogs are anesthetized and positioned in dorsal recumbency.\(^3\) Tibiotarsal joint angles generally are measured in dogs just prior to force measurements (see separate GRMD protocol). The pelvic limb is held so that the hip (coxofemoral) and knee (stifle) joints each forms a 90° angle. The distal limb is supported with a finger placed just below the tibiotarsal joint (hock). The tibia should be parallel to the table. A goniometer is held so that the pivot point is centered over the lateral malleolus of the fibula, with one arm of the goniometer extending along the axis of the tibia/fibula and the other along the metatarsus (Figure 1).

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**Figure 1.** Placement of the goniometer to measure the tibiotarsal joint. The pivot point is centered over the lateral malleolus of the fibula. One arm extends along the axis of the tibia/fibula, with the other along the metatarsus. The angle here is approximately 165°.
5. METHODS

5.1. Anesthetic protocol (Note, in a preliminary study, mean alveolar concentration [MAC] values for isoflurane did not significantly affect force measurement values [Schueler RO, Koch J, Kornegay JN, unpublished data]).

20-30 minutes prior to anesthesia induction:
- Pre-anesthetic agents:
  - Atropine sulfate (0.04 mg/kg, IM)
  - Acepromazine maleate (0.02 mg/kg, IM) for dogs weighing greater than 5 kg
  - Butorphanol tartrate (0.4 mg/kg, IM)

Anesthetic monitoring:
During anesthesia, ECG, heart and respiratory rate, blood pressure, end tidal (Et)CO$_2$, and saturation of hemoglobin by peripheral oxygen (SpO$_2$) are monitored continuously with a pulse oximeter (Cardell Multiparameter Monitor 9405, Minrad International, Inc, Orchard Park, NY). These values, as well as capillary refill time and anesthetic setting, are recorded every 15 minutes.

Anesthetic induction:
- Anesthetic agents:
  - Propofol (up to 3 mg/kg, IV – slowly!) for dogs weighing greater than 5 kg
  - Isoflurane or sevoflurane (to effect, inhaled) (avoid masking down)

Anesthetic recovery:
Monitor affected and carrier dogs closely during anesthetic recovery until fully awake and in sternal recumbency.
- Naloxone (up to 0.4 mg/kg, SQ) for rapid recovery; given in ½ dose increments (1$^{st}$ dose given while the dog is still intubated and breathing O$_2$; 2$^{nd}$ dose, if necessary, after extubated and/or if respiration drops below 7 breaths per minute).

5.2. Position the dog in dorsal recumbency.
5.3. Hold the pelvic limb so that the hip (coxofemoral) and knee (stifle) each forms a 90° angle. The distal limb is supported with a finger placed just below the tibiotalar joint (hock). The tibia should be parallel to the table. A goniometer is held so that the pivot point is centered over the lateral malleolus of the fibula, with one arm of the goniometer extending along the axis of the tibia/fibula and the other along the metatarsus (Figure 1). Note, the paw tends to deviate dorsally from the line of the metatarsus. The arm of the goniometer should be placed along the metatarsus and not aligned with the end of the paw.

5.4. Record the joint angle.
5.5. Repeat the measurement on the other limb.

6. EVALUATION AND INTERPRETATION OF RESULTS

Tibiotarsal joint angle was measured previously in GRMD dogs in the context of serial peroneus longus force measurements.$^{3,4}$ In that study, single pins were transversely placed proximally and distally in the tibia and were secured to metal rods mounted on a heavy plastic base. Dogs were positioned so that the tibia was parallel to the table and perpendicular to the femur. We noticed that the angle formed by the tibiotalar joint often was more acute in affected dogs. Initially, the angle formed by the flexor surface of the tarsus was traced on a transparency and then measured by use of a protractor. Tibiotarsal joint angles of GRMD and normal dogs measured using this method were not statistically different at 3 months of age.$^{3}$ However, by 6 months, GRMD angles were more acute, in keeping with flexor contractures seen when dogs are standing or walking (Figure 1).

Subsequent to this study, so as to be able to compare results with historical values, we have continued to use this basic approach without the need for surgery or immobilization of the limb. An earlier paper found similar values for this angle in standing dogs.$^{5}$ Since our report of tibiotalar contractures in GRMD, others have described methods to measure tibiotalar and other joint angles at maximal flexion and extension in normal dogs.$^{6,7}$ We now measure all pelvic limb angles based on the method of Jaegger et al$^{6}$ to gain a complete assessment. Tibiotarsal joint angles for normal dogs using our method approximate but are somewhat less than those recorded at maximal extension.
Tibiotalar joint angles and force measurements (see separate GRMD protocol) correlate. Correlation coefficients were calculated in a group of 51 GRMD dogs. There was a strong correlation between tibiotalar joint isometric tetanic force and angle. Values (mean ± SD) for extension (2.138 ± 0.915 N/kg) correlated directly (r = 0.54; p < 0.0001; power = 0.987), while those for flexion (0.443 ± 0.132 N/kg) correlated inversely (r = -0.70; p < 0.0001; power = 1.00) with joint angle (148.08 ± 12.84°). Dogs with weak extension and strong flexion force values tended to have tibiotalar joint flexor contractures. Values in heterozygous males and homozygous females do not differ statistically, so both genders can be assessed in parallel during treatment trials. Importantly, by comparing serial measurements from treated and untreated groups, one can document improvement or delayed progression of disease.

**Potential Advantages/Disadvantages of the Methodology**

**Advantages**
Measurement of tibiotalar joint angle is simple and correlates with tibiotalar joint force, suggesting that it should accurately predict overall disease progression.

**Disadvantages**
There is the potential for variable placement of the goniometer by different examiners. Tibiotalar joint angles of normal and GRMD dogs do not differ at 3 months of age. Thus, studies must be extended beyond this time point for them to be useful in demonstrating treatment benefit. While tibiotalar joint angle provides insight on broader postural and joint contractures in GRMD, other angles must be measured to gain a comprehensive assessment.
7. REFERENCES


